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54 Novel protein H being capable of binding to IgG, gene coding for said protein H and a process for producing said protein H.

57 A gene coding for Protein H, which is capable of binding specifically to human IgG of all subclasses, was isolated from Streptococcus sp. AP1 and expressed in host cells, E. coli to produce the Protein H

# NOVEL PROTEIN H BEING CAPABLE OF BINDING TO IgG, GENE CODING FOR SAID PROTEIN H AND A PROCESS FOR PRODUCING SAID PROTEIN H

The present invention relates to a novel protein binding specifically to the Fc fragment of human immunoglobulin G(IgG), a gene coding for said protein and to a process for producing said protein.

It has been known that certain microorganisms produce a series of proteins known as bacterial Fc receptors which have an affinity to the Fc fragment of immunoglobulins (Boyle et al., Bio/Technology 5, 697(1987)).

Typical examples of such proteins are Protein A derived from Staphylococcus aureus and Protein G derived from Streptococcus G148.

These proteins characteristically bind to the Fc fragment of immunoglobulins and are used for assays, the purification and preparation of antibodies as well as clinical diagnosis and biological research.

They can also be used for the treatment of cancer and autoimmune diseases in which the proteins immobilized onto insoluble carriers are used to adsorb or remove undesirable immune complexes from blood(Cancer 46, 675(1980)).

These known proteins have some undesirable properties as agents for purification of human monoclonal antibodies produced from non-human animal cells or for removal of excessive IgG from blood for the purposes of blood purification.

Protein A binds to IgGs of various animal species including human beings as well as human IgA, IgM and so on. Protein G binds only to IgG, but it binds both to human IgG and to IgGs of other animal species (Fahnestock, Trends in Biotechnology 5, 79 (1987)). Thus, their binding specificities are not so narrow that they can be used for assay, purification and adsorption or removal of human IgG.

Under these circumstances, the development of a protein capable of binding specifically to human IgG has been demanded.

It was suspected that a protein which binds to human IgG(IgG1, IgG2, IgG3 and IgG4) but not to IgGs of other animal species would be present in cells of group A Streptococcus strains (Björck, J. Immunol., 133, 969(1984)). However, no such protein has been isolated. Two types of IgG-Fc-binding proteins have been isolated from group A Streptococcus, one of which binds to human IgG(IgG1, IgG2 and IgG4), pig IgG and rabbit IgG; and the other binds specifically to human IgG3 (Boyle et al., Bio/Technology 5, 697(1987)).

It has been unknown whether or not such IgG-Fc-binding protein which binds specifically to human IgG (IgG1, IgG2, IgG3 and IgG4) but which does not bind to IgGs of most other animal species and to human IgA, IgD, IgE and IgM exists and whether or not a sufficient amount of such protein can stably be obtained.

Thus, the technical problem underlying the present invention is to provide proteins which specifically bind to human IgG. The solution thereof is achieved by providing the embodiments characterized in the claims. It is based on the finding that Streptococcus sp. AP1 belonging to group A Streptococcus produces a protein with the above-mentioned properties. The invention also relates to a gene coding for said protein and to a process for producing said protein in which said gene is used.

Thus, the present invention provides a novel protein capable of specifically binding to human IgG, and useful for assay and purification of human IgG, removal or adsorption of excessive IgG from blood and for diagnosis of autoimmune diseases. It also provides a gene coding for said protein and a process for producing the said protein in industrial scales.

The protein provided by the present invention, which is hereinafter referred to as Protein H, is a protein capable of binding to the Fc fragment of immunoglobulins and produced by a strain of group A Streptococcus. It has the following binding specificity:

- i) It binds to human IgG(IgG1, IgG2, IgG3 and IgG4) and rabbit IgG;
- ii) It does not bind to IgGs of mouse, rat, bovine animal, sheep and goat;
- iii) It does not bind to human IgA, IgD, IgE and IgM; or the following binding specificity:
  - i) It strongly binds to human IgG(IgG1, IgG2, IgG3 and IgG4), human IgG-Fc and rabbit IgG;
  - ii) It weakly binds to pig IgG;
  - iii) It does not bind to IgGs of mouse, rat, bovine animal, sheep, goat and horse;
  - iii) It does not bind to human IgGfab, IgA, IgD, IgE and IgM.

The strain, Streptococcus sp. AP1 which produces the Protein H has been deposited at the Fermentation Research Institute, Japan, under the deposit No. FERM-10374, and also under the deposit No. FERM BP-2371 according to the BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE.

The protein H can be produced by the genetic engineering technology using the gene coding for the Protein H.

The Protein H produced from the gene isolated from the *Streptococcus* sp. AP1 has the amino acid sequence given in the Fig. 1. This sequence was identified by the analysis of the DNA sequence of the gene.

It is to be understood that subfragments or variants of the Protein H specifically disclosed in the present application wherein the original amino acid sequence is modified or altered by insertion, addition, substitution, inversion or deletion of one or more amino acids are within the scope of the present invention as far as they retain the essential binding specificity as mentioned above.

The gene coding for the Protein H can be isolated from the chromosomal DNA of a Protein H-producing strain such as *Streptococcus* sp. AP1 based on the information on the DNA sequence of the Protein H shown in Fig. 4. The isolation of the gene can also be carried out as follows:

The chromosomal DNA can be isolated from cells of the Protein H-producing strain in accordance with known methods (Fahnestock, J. Bacteriol. 167, 870 (1986)). The isolated chromosomal DNA is then segmented into fragments of adequate lengths by biochemical means such as digestion with a restriction enzyme or physical means.

The resulting fragments are then inserted at an adequate restriction site into an adequate cloning vector such as  $\lambda$ gt11 (Young et al., Proc. Natl. Acad. Sci. USA 80, 1194 (1983)) or plasmid vectors such as pUC18 (Messing et al., Gene 33, 103 (1985)).

The vectors are then incorporated into adequate host cells such as *E. coli*.

From the resulting transformants, the clones producing the protein which binds to human IgG or the Fc region of human IgG are selected by a known method (Fahnestock et al., J. Bacteriol., 167, 870 (1986)).

After the proteins capable of binding to human IgG or the Fc region of human IgG are isolated from the resulting positive clones according to conventional methods, the binding specificities of the proteins are determined to select the clones producing Protein H. Fig. 2 shows the binding specificity of Protein H.

After the DNA insert of said clone is isolated by conventional methods, the DNA sequence of the insert is determined by known methods (Sanger et al., Proc. Natl. Acad. Sci. USA 74, 5463 (1977); Choen et al., DNA 4, 165 (1985)). Fig. 4 shows the DNA sequence of the DNA insert isolated from the positive clone Fc4. Fig. 5 shows the DNA sequence of the structural gene coding for the Protein H isolated from said clone.

The invention also relates to DNA sequences that hybridize with said identified DNA sequence under conventional conditions and that encode a protein displaying essentially the same binding properties as said protein H. In this context the term "conventional conditions" refers to hybridization conditions where the  $T_m$  value is between about  $T_m-20$  and  $T_m-27$ . Stringent hybridization conditions are preferred.

It is necessary for genes to be expressed that they contain expression-control regions such as promoter, terminator and the like. The gene shown in Fig. 4 contains such necessary expression-control regions.

The expression of genes may be effected with expression vectors having the necessary expression control regions in which only the structural gene is inserted. For this purpose, the structural gene shown in Fig. 5 can preferably be used. The structural gene coding for the Protein H can be obtained from the DNA sequence of Fig. 4 or synthesized by conventional methods based on the amino acid sequence given in the present specification.

As for the expression vectors, various host-vector systems have already been developed, from which the most suitable host-vector systems can be selected for the expression of the gene of the present invention.

It has been known that, for each host cell, there is a particularly preferred codon usage for the expression of a given gene. In constructing a gene to be used for a given host-vector system, the codons preferred by the host should be used. Adequate sequences for the gene for the Protein H to be used in a particular host-vector system can be designed based on the amino acid sequence given in Fig. 1 and synthesized by conventional synthetic methods.

The present invention further relates to a process for producing the Protein H by culturing a host cell transformed with an expression vector into which the gene encoding the Protein H is inserted.

The process comprises steps of

- i) inserting a gene coding for the Protein H into a vector;
- ii) introducing the resulting vector into a suitable host cell;
- iii) culturing the resulting transformant cell to produce the Protein H; and
- iv) recovering the Protein H from the culture.

In the first step, the gene coding for the Protein H, which is isolated from the chromosomal DNA of *Streptococcus* sp. AP1 or synthesized as mentioned above, is inserted into a vector suitable for a host to be used for the expression of the Protein H. The insertion of the gene can be carried out by digesting the vector with a suitable restriction enzyme and linking thereto the gene by a conventional method.

In the second step, the resulting vector with the gene is introduced into host cells. The host cells may be *Escherichia coli*, *Bacillus subtilis* or *Saccharomyces cerevisiae* and the like. The introduction of the expression vector into the host cells can be effected in a conventional way.

In the third step, the resulting transformant cells are cultured in a suitable medium to produce the Protein H by the expression of the gene. The cultivation can be conducted in a conventional manner.

In the fourth step, the produced Protein H is recovered from the culture and purified, which can be conducted by known methods. For example, the cells are destroyed by known methods such as ultrasonification, enzyme treatment or grinding. The Protein H released by the cells or secreted into the medium is recovered and purified by conventional methods usually used in the field of biochemistry such as ion-exchange chromatography, gel filtration, affinity chromatography using IgG as ligand, hydrophobic chromatography or reversed phase chromatography, which may be used alone or in suitable combinations.

As mentioned above, the protein provided by the present invention can be used for identification or separation of human IgG. For these purposes, the protein may be brought into a reagent kit or a pharmaceutical composition by mixing or combining it with suitable reagents, additives or carries.

The present invention will more precisely be described by the following examples. But, they are not intended to limit the scope of the present invention.

In the attached drawings, Fig. 1 represents the amino acid sequence of the Protein H. Fig. 2 gives autoradiograms showing binding specificities of Protein G and Protein H to various antibodies. Fig. 3 is a diagram schematically illustrating the DNA inserts of clones Fc4 and Fc16. Fig. 4 gives the DNA sequence of the DNA insert in clone Fc4. Fig. 5 gives the DNA sequence of the structural gene of the Protein H. Fig. 6 illustrates plasmid pPH-1 and its deletion plasmids used for the determination of the DNA sequence. Fig. 7 is a graph showing the binding activity of *Streptococcus* sp. AP1 to human IgG, IgGFc and mouse IgG. Fig. 8 gives autoradiograms showing binding specificities of Protein H purified from the periplasmic fraction of *E. coli* JM109 (pPH-1) and Protein G to various antibodies.

#### Example 1. IgG-binding activity of *Streptococcus* sp. AP1

To an Eppendorf tube was added 20  $\mu$ l of dichloromethane solution of IODO-GENTM (1,3,4,6-tetrachloro-3 $\alpha$ , 6 $\alpha$ -diphenyl glycouril; Pierce and Warriner Ltd.; 0.1 mg/ml), which was dried by blowing nitrogen gas in the tube, while the tube was inclined and rotated. To the resultant, 200  $\mu$ l of a buffer solution A (50mM Na-phosphate buffer pH 7.5, 0.01% Pluronic F-68 (BASF Corp.)) is added. After the mixture was allowed to stand in an ice bath for 10 minutes, the buffer was removed. To the tube were added 10  $\mu$ l of 0.5M Na-phosphate buffer (pH 7.5) and 25  $\mu$ l of IgG solution (Human IgG 5  $\mu$ g, mouse IgG 5  $\mu$ g, human IgGFc 3.34  $\mu$ g; Cappel Laboratories), followed by 2  $\mu$ l of Na<sup>125</sup>I solution (IMS 30, Carrier-free, 100 mCi/ml; Amersham Corp.). The mixture was allowed to stand for 15 minutes, while the mixture was ice-cooled and softly shaken. The reaction product was transferred to a serum tube containing 200  $\mu$ l of a buffer solution (10mM Na-phosphate buffer pH 7.2, 150mM NaCl) after the serum tube had been treated with the buffer solution A (5 ml) in the same way as above-mentioned. The mixture was allowed to stand in an ice bath for 5 minutes. The resulting solution was applied to a PD-10 column (Pharmacia Fine Chemicals) equilibrated with buffer solution B (30mM Na-phosphate buffer pH 7.3, 120mM NaCl, 0.1 % BSA) and eluted with buffer solution B. From each fraction (0.5 ml), 2  $\mu$ l was sampled and measured with a  $\gamma$ -counter. (Ria Gamma "QUATRO"; LKB Corp.) to recover the <sup>125</sup>I-labeled IgG.

Thus, <sup>125</sup>I-human IgG  $2.24 \times 10^7$  cpm/ $\mu$ g ( $1.12 \times 10^8$  cpm/ml), <sup>125</sup>I-human IgGFc  $8.98 \times 10^7$  cpm/ $\mu$ g ( $3 \times 10^8$  cpm/ml) and <sup>125</sup>I-mouse IgG  $2.42 \times 10^7$  cpm/ $\mu$ g ( $1.21 \times 10^8$  cpm/ml) were obtained.

A loopful of cells of *Streptococcus* sp. AP1 was inoculated to 5 ml of Todd-Hewitt culture medium (Difco Laboratories) and incubated at 37°C for 10 hours. Of the culture solution, a 2 ml-portion was added to 100 ml of Todd-Hewitt medium, incubated at 37°C for 13 hours, and centrifuged to harvest the cells.

The cells were washed with 100 ml of buffer solution C (30mM Na-phosphate buffer pH 7.2, 120mM NaCl, 0.05 % Tween 20, 0.02% NaN<sub>3</sub>) and diluted with buffer solution C to give suspensions of different cell concentrations between 10<sup>7</sup> to 10<sup>10</sup> cfu/ml. In a serum-tube were added a 200  $\mu$ l-portion of each suspension, followed by <sup>125</sup>I-labeled IgG (human IgG 10 ng, human IgGFc 5.2 ng, mouse IgG 10 ng), and the mixture was stirred and allowed to stand at 37°C still for 2 hours. After the reaction has completed, 2 ml of buffer solution C was added and centrifuged to harvest the cells. After similar retreatment of the cells with 2 ml of buffer solution C, the amount of <sup>125</sup>I-labeled IgG bound to cells was measured with a  $\gamma$ -counter.

As the results in Fig. 7 show, *Streptococcus* sp. AP1 cell has proved to bind to human IgG and IgGFc but not to mouse IgG.

Example 2. Preparation of chromosomal DNA of Streptococcus sp. AP1

A loopful of cells of Streptococcus sp. AP1 was inoculated to 10 ml of Todd-Hewitt culture medium and cultivated at 37° C for 13 hours. Of the culture, a 8 ml-portion was added to 400 ml of Todd-Hewitt medium, and cultivated at 37° C for 3 hours ( $A_{660} = 0.6$ ). After 22 ml of 10 % cysteine and 26 ml of 0.4M DL-Threonine were added, the culture was again incubated for one hour. Then 250 ml of 15 % glycine was added and cultivation was continued additionally for 2 hours. Cells were harvested by centrifugation and washed with 0.2M sodium acetate. The washed cells were suspended in 40 ml of buffer solution D (0.15M NaCl, 0.015M  $\text{Na}_3\text{-citrate}$  pH 7.4-7.6) containing 27% sucrose and 10 mM EDTA. To the suspension, 2500 units of Mutanolysin (Sigma Chemical Co.) was added and incubated at 37° C for 3 hours. To the reaction mixture, 4 ml of 10 % SDS and proteinase K (0.2 mg/ml) were added and incubated overnight at room temperature. After the extractions with phenol followed by ether, twice volume of cold ethanol were added to the removed water phase and the separated DNA was recovered by winding it around a glass rod.

The recovered DNA was dissolved in 5 ml of buffer solution D and incubated with RNase A (100  $\mu\text{g/ml}$ ) at 37° C for 1 hour. Then phenol extraction and ethanol precipitation were carried out to recover DNA.

Yield of chromosomal DNA amounted to about 1 mg.

Example 3. Cloning of Protein H gene

The chromosomal DNA (about 100  $\mu\text{g}$ ) obtained in Example 2 was dissolved in 200  $\mu\text{l}$  of a buffer solution comprising 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA and passed through a needle (27G) for use in injection to shear the DNA fragments of 2 to 10 kb. About 10  $\mu\text{g}$  of the obtained DNA fragments was added to a solution comprising 40 mM Tris-HCl (pH 7.5) 5 mM DDT, 10 mM  $\text{MgCl}_2$ , 0.1 mg/ml BSA, 50  $\mu\text{M}$  dNTP, (dATP, dTTP, dGTP, dCTP) and 10 units of T4DNA polymerase and allowed to react at 24° C for 2 hours to make them blunt-ended. Then phenol extraction and ethanol precipitation were carried out, and the thus-collected blunt ended DNA fragments were added to 50  $\mu\text{l}$  of a solution comprising 100 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 80  $\mu\text{M}$  S-adenosylmethionine and 200 units of EcoRI methylase and allowed to react at 37° C for 20 minutes to make them methylated. Then phenol extraction and ethanol precipitation were carried out, and the thus-collected methylated DNA fragments were reacted at 16° C for 12 hours with a commercially-available EcoRI linker which had already been phosphorylated by the use of a commercially available ligation kit (Takara Shuzo Co., Ltd., Japan). The resulting reaction product was added to a solution comprising buffer E (10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 10 mM  $\text{MgCl}_2$ , 1 mM DTT) and 200 units of EcoRI, and allowed to react at 37° C for 12 hours.

After termination of this reaction, phenol extraction and isopropanol precipitation were carried out to collect the DNA.

The thus-obtained DNA (about 0.5  $\mu\text{g}$ ) was reacted at 16° C for 16 hours with 1  $\mu\text{g}$  of  $\lambda\text{gt11}$  DNA (Proclone™  $\lambda\text{gt11}$  system: Promega Biotech Corp.) in 13  $\mu\text{l}$  of a solution comprising buffer F (66 mM Tris-HCl pH 7.6, 6.6 mM  $\text{MgCl}_2$ , 10 mM DTT, 0.1 mM ATP) and 400 units of T4 DNA ligase.

The ligated DNAs were packaged into phage using in vitro packaging kit (Gigapack Gold; Stratagene Corp.) and used as gene library of Streptococcus sp. AP1. The packaging efficiency, as measured with E. coli Y1090, was  $3.2 \times 10^6$  pfu/ $\mu\text{g}$   $\lambda\text{gt11}$  DNA.

E. coli Y1090 was cultivated in LBM medium {LB medium (Bacto tryptone 1 %, Yeast extract 0.5 %, NaCl 0.5 %; pH 7.2), 10 mM  $\text{MgSO}_4$ , 0.2 % maltose, 50  $\mu\text{g/ml}$  Ampicillin} to grow up to  $A_{660} = 0.6$ . From this culture, 0.2 ml was collected and centrifuged to harvest cells.

The cells were suspended in 0.2 ml of buffer solution G (10 mM Tris-HCl pH 7.4, 10 mM  $\text{MgSO}_4$ , 0.01 % gelatin), mixed with 100  $\mu\text{l}$  of buffer solution G and 7.6  $\mu\text{l}$  of the gene library phage particles solution ( $5 \times 10^4$  pfu), and incubated at 37° C for 20 minutes. To this reaction mixture, 7 ml of soft agar solution (LBM medium, 0.4 % soft agar, 47° C) was added, stirred, and overlaid onto a LBM plate (diameter 150 mm). After 3 hours' incubation at 42° C, the plate was covered with nitrocellulose filter (BA 85, diameter 142 mm; Schleicher and Schuell AG) which had been immersed in 20 mM IPTG solution for 5 minutes and dried, and incubated at 37° C for 16 hours.

After the cultivation has been completed, nitrocellulose filter was taken off. Then procedure was proceeded at room temperature with slow shaking as follows:

The nitrocellulose filter was treated in 50 ml of buffer solution H (10 mM Veronal buffer pH 7.4, 0.15 M NaCl) for 5 minutes, and incubated for 1 hour in 50 ml of buffer solution H containing 0.25 % gelatin and 0.25 % Tween 20. After 3 hour incubation with human IgGfc fragment (2  $\mu\text{g/ml}$ ) (CAPPEL Corp.) in 40 ml of buffer solution H containing 0.1 % gelatin, the filter was washed three times with 40 ml each of buffer

solution H containing 0.1 % gelatin for 10 minutes. Again 1 hour incubation with goat anti-human IgGFC (Peroxidase conjugate, affinity purified; Jackson Immunoresearch Laboratories Corp.; diluted 1,000 fold with buffer solution H containing 0.1 % gelatin) in 40 ml of buffer solution H containing 0.1 % gelatin was carried out, and the filter was washed with 40 ml of buffer solution H containing 0.1 % gelatin three times each for 10 minutes, followed by 40 ml of buffer solution K (20 mM Tris·HCl pH 7.5, 0.5 M NaCl) once for 5 minutes. This filter was immersed in a color-developing solution (20 mg 4-chloro-1-naphthol/6.6 ml methanol, 20  $\mu$ l H<sub>2</sub>O<sub>2</sub>/33.4 ml buffer solution K) for 30 minutes. Out of 70,000 plaques, 17 purple-plaques were collected and suspended in 500  $\mu$ l of buffer solution G. To the suspension, 10  $\mu$ l of chloroform was added and allowed to stand for 40 minutes, and then centrifuged (10,000 rpm, 1 minute). The same procedure with the resulting supernatant was repeated to give stable clones Fc4 and Fc16.

The IgGFC-binding proteins produced by clone Fc4 and Fc16 had the same apparent molecular weight of 45 kDa as judged by the Western-blotting method.

Phage solutions of Fc4 and Fc16 (400  $\mu$ l, about 10<sup>10</sup> pfu/ml) each was mixed with culture of *E. coli* Y1090 (400  $\mu$ l), to which soft agar solution (8 ml) was added. The resulting mixture was laid over 8 sheets of LB plates. After incubation at 37 °C for 16 hours, 15 ml of buffer solution M (50 mM Tris·HCl pH 7.5, 100 mM NaCl, 8.1 mM MgSO<sub>4</sub>, 0.01 % gelatin) was added every plate and shaken at 4 °C for 3 hours.

After the buffer solutions were collected, the plates were washed with buffer (2 ml/plate), which was then combined with the collected buffer solution. To the combined buffer solution, 2 ml of chloroform was added and the mixture was stirred and centrifuged at 7,000 rpm for 15 minutes.

The supernatant was again centrifuged (17,000 rpm; 3 hours). The recovered precipitate was suspended in 0.5 ml of buffer solution M, to which CsCl was added to a concentration of 0.5 mg/ml. The suspension was then centrifuged (22,000 rpm; 2 hours, 4 °C) to recover phage particles. The phage particle suspension was dialyzed against a buffer solution comprising 50 mM Tris·HCl, pH 8.0, 10 mM NaCl, and 10 mM MgCl<sub>2</sub>. To the dialyzed solution, EDTA (final concentration 20 mM), SDS (final concentration 0.5 %) and proteinase K (final concentration 50  $\mu$ g/ml) were added, and the mixture was incubated at 65 °C for 1 hour, and extracted with phenol followed by chloroform.

The aqueous phase was dialyzed against a buffer solution comprising 10 mM Tris·HCl, pH 8.0, and 1 mM EDTA, and precipitated with ethanol to give DNA.

The obtained phage DNA (200  $\mu$ g) was dissolved in 200  $\mu$ l of a buffer solution comprising 10 mM Tris·HCl, pH 7.5, and 1 mM EDTA, and the restriction enzyme cleavage pattern was analyzed to find that clones Fc4 and Fc16 had respective DNA inserts as shown in Fig. 3.

*E. coli* Y1089 was lysogenized with phage clones Fc4 and Fc16 according to the methods described by Young et al. (Proc. Natl. Acad. Sci. USA 80, 1194 (1983)).

#### Example 4. Binding specificity of Protein H

*E. coli* Y1089 lysogenized with phage Fc4 was inoculated to 40 ml of the aforesaid LBM medium and incubated at 28 °C for 16 hours. The seed culture was added to 2 liters of LBM medium and incubated at 28 °C for 145 minutes.

To the culture, IPTG was added to a final concentration of 1 mM and incubated at 42 °C for 45 minutes, and at 37 °C for additional 1 hour. The cells were harvested by centrifugation and suspended in 100 ml of a buffer solution comprising 50 mM phosphate buffer (pH 7.2), 5 mM EDTA, 5 mM benzamidine·HCl and 5 mM iodoacetamide and subjected to 10 minute ultrasonification. The mixture was centrifuged at a low speed to remove cell debris, and at 50,000 rpm for 30 minutes. The supernatant was applied to a IgG-Sepharose (6 Fast Flow; Pharmacia.) column (10 ml) which had been successively washed with 400 ml of buffer solution N (50 mM Tris·HCl pH 7.6, 150 mM NaCl, 0.05 % Tween 20), 2.5 M NaI (pH 7.2) and buffer solution N, and equilibrated with buffer solution N. After washing the column with 300 ml of buffer solution N, elution for recovering protein H was carried out with 40 ml of 2.5 M NaI (pH 7.2).

Fractions of 0.5 ml each were collected and a small amount of sample collected from each fraction was spotted on a nitrocellulose filter. Then detection of Protein H containing fractions was carried out according to the staining method described in Example 3.

The Protein H-containing fractions were combined and dialyzed once against 1 liter of buffer solution comprising 50 mM phosphate buffer (pH 7.2), 0.15 M NaCl and 0.25 % NaI and twice against 5 liters each of buffer solution comprising 50 mM phosphate buffer (pH 7.2) and 0.15 M NaCl, and then concentrated to about 1 ml with Amicon YM-5 (Amicon Corp.). The concentrated solution was applied to a gel filtration column for HPLC (diameter 7.5 mm x 6 cm, TSK gel G-3000 SW (Toyo Soda Co., Ltd.)) equilibrated with a buffer solution comprising 50 mM phosphate buffer (pH 7.5) and 0.2 M NaCl, and eluted with the same

buffer solution at a flow rate of 0.4 ml/min. The fractions collected between the 34th to 36th minute of elution, which contains Protein H, were combined and concentrated with Amicon YM-5. The concentrated solution was applied to a reversed phase HPLC column (diameter 4.6 mm x 7.5 cm, TSK gel Phenyl-5PW RP (Toyo Soda Co., Ltd.)) equilibrated with a buffer solution comprising 0.1 M glycine/NaOH (pH 10.0) and 1 mM tetra-n-butyl ammonium hydroxide, and the Protein H was eluted with a linear gradient (0 % → 66 % , 2 %/min) of acetonitrile. Fractions collected near the 16th minute of elution, which contains Protein H, were combined and concentrated to about 2 ml under reduced pressure. The concentrated solution was applied to a PD-10 column (Pharmacia Corp.) equilibrated with water, and eluted with water to remove salts. Of the obtained protein, yield amounts to about 53 µg and molecular weight was about 45 kDa as measured by the Western-blotting technique using the staining method described in Example 3.

About 10 µg of Protein G (Genex Corp.) and about 10 µg of the aforesaid Protein H were labeled with Na<sup>125</sup>I according to the method described in Example 1 to give  $1.28 \times 10^7$  cpm/µg ( $8.5 \times 10^7$  cpm/ml) of <sup>125</sup>I-Protein G and  $1.68 \times 10^7$  cpm/µg ( $1.4 \times 10^8$  cpm/ml) of <sup>125</sup>I-Protein H.

Human IgG1, IgG2, IgG3 and IgG4 (all, Protogen Corp.); human IgM, IgG and serum IgA, and IgGs of sheep, rabbit, bovine, and goat (all, Cappel Corp.); human IgD and IgE (all, Serotec Corp.); rat IgG (Jackson Immunoresearch Corp.); mouse IgG (Zymed Corp.) and human monoclonal IgG were dissolved in buffer solution K, and diluted with buffer solution K to concentrations of 0.08 to 10 µg/200 µl. Each diluted solution (200 µl) was applied to nitrocellulose filter (Schleicher and Schuell Corp.) and adsorbed on the filter with BIO-DOT (BioRad Laboratories). The filter was incubated in 40 ml of a buffer solution comprising buffer K, 0.25 % gelatin and 0.25 % Tween-20 at 42 °C for 1.5 hours and washed twice in buffer solution K containing 0.1 % gelatin at room temperature for 15 minutes. The washed filter was further incubated in 40 ml of a solution comprising buffer solution K, 0.1 % gelatin and 0.5 µg ( $1.6 \times 10^5$  cpm/ml) of <sup>125</sup>I-Protein G or 0.5 µg ( $2.1 \times 10^5$  cpm/ml) of <sup>125</sup>I-Protein H at room temperature for 3 hours.

The filter was incubated 4 times with 40 ml of a solution comprising buffer solution K, 0.25 % gelatin, 0.25 % Tween-20 and 0.85 M NaCl at room temperature for 15 minutes for washing. After drying the filter, antibody-binding properties of Protein G and Protein H were analyzed by autoradiography.

The autoradiograms shown in Fig. 2 demonstrated that Protein H having the specificity of

- i) binding to human IgG (IgG1, IgG2, IgG3 and IgG4) and rabbit IgG, and
- ii) not binding to mouse, rat, bovine, sheep and goat IgG's and human IgA, IgD, IgE and IgM.

#### Example 5. The nucleotide sequence of Protein H gene

The phage DNA (about 10 µg) of clone Fc4 obtained in Example 3 was incubated in 100 µl of a solution comprising buffer solution P (10 mM Tris·HCl, pH 7.5, 10 mM MgCl<sub>2</sub>, 1 mM DTT), 30 units of Sac I and 42 units of KpnI at 37 °C for 5 hours. After termination of the reaction, phenol extraction and ethanol precipitation were carried out to recover phage DNA. On the other hand, plasmid pUC 18 (about 8 µg) was incubated in 30 µl of a solution comprising buffer solution P, 20 units of SacI and 14 units of KpnI at 37 °C for 10 hours. Subsequently phenol extraction and ethanol precipitation were conducted to recover DNA. The recovered DNA was dissolved in 50 µl of 1 M Tris·HCl (pH 8.0) and incubated with 0.36 units of Bacterial Alkaline phosphatase (BAP) at 65 °C for 30 minutes. After 0.36 units of BAP was added, the reaction mixture was again incubated at 65 °C for further 30 minutes. Subsequently phenol extraction and ethanol precipitation were carried out to recover DNA.

The BAP-treated pUC18 (0.5 µg) and the phage DNA digested with SacI and KpnI (0.1 µg) were incubated in 30 µl of a solution comprising buffer E and 10 units of T4 DNA ligase at 16 °C for 16 hours. With this reaction mixture, *E. coli* JM109 cells were transformed and ampicillin-resistant transformants were obtained, from which plasmid DNAs were prepared. By analysis of restriction enzyme cleavage pattern, a transformant containing a plasmid pPH-1 as shown in Fig. 6 was selected.

Plasmid pPH-1 (about 10 µg) was incubated in 25 µl of a solution comprising buffer E, 12 units of BamHI and 12 units of PstI at 37 °C for 8 hours. Then the resulting DNA was recovered by phenol extraction and ethanol precipitation, and treated with Deletion kit for Kilo- Sequence (Takara Shuzo Co., Ltd.). *E. coli* JM109 cells were transformed with the DNA and ampicillin-resistant transformants were obtained, from which plasmid DNAs were prepared. Subsequently by the analysis of the restriction enzyme cleavage pattern, transformants containing deletion plasmids shown in Fig. 6 were selected out.

The deletion plasmids and pPH-1 (about 3 µg each) were dissolved in 20 µl each of a solution comprising 2 µl of 2N NaOH and 2 µl of 2 mM EDTA and denaturated at room temperature for 5 minutes. The DNA was recovered by ethanol precipitation and the nucleotide sequence was determined with SEQUENASE (U.S. Biochemical), [ $\alpha$ -<sup>32</sup>P] dCTP (800 Ci/m mole: Amersham Co., Ltd.) and Primer M3

(Takara Shuzo Co., Ltd.).

The nucleotide sequence of the DNA fragment derived from the chromosomal DNA of *Streptococcus* sp. AP1 is as illustrated in Fig. 4. The DNA fragment contains promoter region, SD sequence, and the Protein H-structural gene coding for the amino acid sequence consisting of 376 amino acids (including Met at start point) starting from initiation codon ATG and terminating with termination codon TAA.

The structural gene encodes the amino acid sequence consisting of 376 residues beginning with Met and terminating with Asn, as shown in Fig. 5. The N-terminal amino acid sequence consisting of 41 residues beginning with Met and terminating with Ala has common characteristics to those of the signal sequence considered to be necessary for the protein secretion of gram positive bacteria, and therefore it can be considered that mature Protein H is a protein having an amino acid sequence consisting of 335 residues beginning with Glu and terminating with Asn.

#### EXAMPLE 6. Expression of pPH-1

The *E. coli* JM109 (pPH-1) obtained in Example 5, was cultivated in LB medium containing ampicillin at a concentration of 50 µg/ml at 37°C for 16 hours. The culture was added to 2 liters of the same medium, incubated at 37°C for 4.5 hours, and centrifuged.

The periplasmic fraction was prepared by the cold osmotic shock procedure (Nossal et al., J. Biol. Chem. 241, 3055 (1966)). A mixture of the cytoplasmic and membrane fractions was prepared by sonicating the pellet obtained after cold osmotic shock.

In this procedure, more than 95 % of the β-galactosidase activity was observed in the mixture of cytoplasmic and membrane fractions, while more than 95 % of the β-lactamase activity was observed in the periplasmic fraction.

Both fractions were analysed by the Western-blotting method described in Example 3. The Protein H having an apparent molecular weight of 45 kDa was demonstrated only in the mixture of cytoplasmic and membrane fractions, while the Protein H having an apparent molecular weight of 42 kDa was demonstrated in the periplasmic fraction.

#### Example 7. Binding properties of Protein H purified from the periplasmic fraction of *E. coli* JM109 (pPH-1)

The Protein H having an apparent molecular weight of 42 kDa was purified from the periplasmic fraction obtained in Example 6 by the successive chromatography of IgG-Sepharose and gel filtration according to the methods described in Example 4. Yield of the Protein H amounted to about 4 mg.

The N-terminal amino acid sequence of purified protein was determined by amino acid sequencer (Applied Biosystems model 477A amino acid sequencer; Applied Biosystems Corp.) to be Glu-Gly-Ala-Lys-Ile-Asp-Trp-Gln-Glu-Glu, which was identical to the putative N-terminal amino acid sequence of nature Protein H as described in Example 5.

The purified Protein H was radiolabeled according to the methods described in Example 1. The binding properties of radiolabeled Protein H were determined according to the methods described in Example 4. In addition to immunoglobulins described in Example 4, binding to human IgG<sub>1</sub> and human IgG<sub>2</sub> (all, Cappel Corp.); and horse and pig IgG (all, Cooper Corp.) were also determined.

The autoradiograms shown in Fig. 8 demonstrated that Protein H having the specificity of

- i) binding strongly to human IgG (IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub> and IgG<sub>4</sub>), human IgG<sub>1</sub> and rabbit IgG;
- ii) binding weakly to pig IgG;
- iii) not binding to IgGs of mouse, rat, bovine, sheep, goat and horse; and
- iv) not binding to human IgA, IgD, IgE and IgM.

#### Claims

1. A protein produced by a strain belonging to Group A *Streptococci* and having the following properties:

- i) capable of binding to the Fc fragment of immunoglobulins;
- ii) capable of binding to human IgG (IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub> and IgG<sub>4</sub>) and rabbit IgG;
- iii) incapable of binding to IgGs of mouse, rat bovine animal, sheep and goat;
- iv) incapable of binding to human IgA, IgD, IgE and IgM.



2. A protein produced by a strain belonging to Group A Streptococci and having the following properties:

- i) capable of binding to the Fc fragment of immunoglobulins;
- ii) capable of binding strongly to human IgG (IgG1, IgG2, IgG3 and IgG4), human IgGFc and rabbit  
5 IgG;
- iii) capable of binding weakly to pig IgG;
- iv) incapable of binding to IgGs of mouse, rat, bovine animal, sheep, goat and horse;
- v) incapable of binding to human IgGfab, IgA, IgD, IgE and IgM.

3. The protein according to Claim 1 or 2 wherein said strain is Streptococcus sp. AP1 (FERM BP-2371).

10 4. The protein according to Claim 3 which displays the following amino acid sequence or a subfragment or derivative thereof with the same binding properties:

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10  
 MET THR ARG GLN GLN THR LYS LYS ASN TYR SER LEU ARG LYS LEU  
 20 30  
 5 LYS THR GLY THR ALA SER VAL ALA VAL ALA LEU THR VAL LEU GLY  
 40  
 ALA GLY PHE ALA ASN GLN THR THR VAL LYS ALA GLU GLY ALA LYS  
 50 60  
 10 ILE ASP TRP GLN GLU GLU TYR LYS LYS LEU ASP GLU ASP ASN ALA  
 70  
 LYS LEU VAL GLU VAL VAL GLU THR THR SER LEU GLU ASN GLU LYS  
 80 90  
 15 LEU LYS SER GLU ASN GLU GLU ASN LYS LYS ASN LEU ASP LYS LEU  
 100  
 SER LYS GLU ASN GLN GLY LYS LEU GLU LYS LEU GLU LEU ASP TYR  
 110 120  
 20 LEU LYS LYS LEU ASP HIS GLU HIS LYS GLU HIS GLN LYS GLU GLN  
 130  
 GLN GLU GLN GLU GLU ARG GLN LYS ASN GLN GLU GLN LEU GLU ARG  
 140 150  
 25 LYS TYR GLN ARG GLU VAL GLU LYS ARG TYR GLN GLU GLN LEU GLN  
 160  
 LYS GLN GLN GLN LEU GLU THR GLU LYS GLN ILE SER GLU ALA SER  
 170 180  
 30 ARG LYS SER LEU SER ARG ASP LEU GLU ALA SER ARG ALA ALA LYS  
 190  
 LYS ASP LEU GLU ALA GLU HIS GLN LYS LEU GLU ALA GLU HIS GLN  
 200 210  
 35 LYS LEU LYS GLU ASP LYS GLN ILE SER ASP ALA SER ARG GLN GLY  
 220  
 LEU SER ARG ASP LEU GLU ALA SER ARG ALA ALA LYS LYS GLU LEU  
 230 240  
 40 GLU ALA ASN HIS GLN LYS LEU GLU ALA GLU HIS GLN LYS LEU LYS  
 250  
 GLU ASP LYS GLN ILE SER ASP ALA SER ARG GLN GLY LEU SER ARG  
 260 270  
 45 ASP LEU GLU ALA SER ARG ALA ALA LYS LYS GLU LEU GLU ALA ASN  
 280  
 HIS GLN LYS LEU GLU ALA GLU ALA LYS ALA LEU LYS GLU GLN LEU  
 290 300  
 50 ALA LYS GLN ALA GLU GLU LEU ALA LYS LEU ARG ALA GLY LYS ALA  
 55

310  
 SER ASP SER GLN THR PRO ASP THR LYS PRO GLY ASN LYS ALA VAL  
 320 330  
 PRO GLY LYS GLY GLN ALA PRO GLN ALA GLY THR LYS PRO ASN GLN  
 5  
 340  
 ASN LYS ALA PRO MET LYS GLU THR LYS ARG GLN LEU PRO SER THR  
 350 360  
 GLY GLU THR ALA ASN PRO PHE PHE THR ALA ALA ALA LEU THR VAL  
 10  
 370  
 MET ALA THR ALA GLY VAL ALA ALA VAL VAL LYS ARG LYS GLU GLU  
 15  
 ASN

5. The protein according to Claim 4 which is the subfragment starting from the forty-second amino acid (GLU) and ending at the last amino acid of the amino acid sequence of Claim 4 or a derivative or subfragment thereof having the same binding properties.

6. A DNA sequence coding for the protein of any one of Claims 1 to 5.

7. The DNA sequence according to Claim 6 which is the following DNA sequence.

10 20 30 40 50  
 25 ATGACTAGAC AACAAACCAA GAAAAATTAT TCACTACGGA AACTAAAAAC  
 60 70 80 90 100  
 CGGTACGGCT TCAGTAGCCG TTGCTTTGAC CGTTTTGGGC GCAGGTTTTG  
 110 120 130 140 150  
 30 CAAACCAAAC AACAGTTAAG GCGGAAGGGG CTAAAATTGA TTGGCAAGAA  
 160 170 180 190 200  
 GAGTATAAAA AGTTAGACGA AGATAATGCT AAAGTTGTTG AGGTTGTTGA  
 210 220 230 240 250  
 35 AACCACAAGT TTGGAACG AAAAACTCAA GAGTGAGAAT GAGGAGAATA  
 260 270 280 290 300  
 AGAAAAATTT AGACAACTT AGCAAAGAAA ATCAAGGAAA GCTCGAAAAA  
 310 320 330 340 350  
 40 TTGGAGCTTG ACTATCTCAA AAAATTAGAT CACGAGCACA AAGAGCACCA  
 360 370 380 390 400  
 AAAAGAACAA CAAGAACAAG AAGAGCGACA AAAAAATCAA GAACAATTAG

410 420 430 440 450  
 AACGTAAATA CCAACGAGAA GTAGAAAAAC GTTATCAAGA ACAACTCCAA  
 460 470 480 490 500  
 AAACAACAAC AATTAGAAAC AGAAAAGCAA ATCTCAGAAAG CTAGTCGTAA  
 510 520 530 540 550  
 GAGCCTAAGC CGTGACCTTG AAGCGTCTCG TGCAGCTAAA AAAGACCTTG  
 560 570 580 590 600  
 AAGCTGAGCA CCAAAAACTT GAAGCTGAGC ACCAAAACTT TAAAGAAGAC  
 610 620 630 640 650  
 AAACAAATCT CAGACGCAAG TCGTCAAGGC CTAAGCCGTG ACCTTGAAGC  
 660 670 680 690 700  
 GTCTCGTGCA GCTAAAAAAG AGCTTGAAGC AAATCACCAA AAACCTGAAG  
 710 720 730 740 750  
 CTGAGCACCA AAAAATTAAA GAAGACAAAC AAATCTCAGA CGCAAGTCGT  
 760 770 780 790 800  
 CAAGGCCTAA GCCGTGACCT TGAAGCGTCT CGTGCAGCTA AAAAAGAGCT  
 810 820 830 840 850  
 TGAAGCAAAT CACCAAAAAC TTGAAGCAGA AGCAAAAGCA CTCAAAGAAC  
 860 870 880 890 900  
 AATTAGCGAA ACAAGCTGAA GAACTTGCAA AACTAAGAGC TGGAAAAGCA  
 910 920 930 940 950  
 TCAGACTCAC AAACCCCTGA TACAAAACCA GGAAACAAAG CTGTTCCAGG  
 960 970 980 990 1000  
 TAAAGGTCAA GCACCACAAG CAGGTACAAA ACCTAACCAA AACAAAGCAC  
 1010 1020 1030 1040 1050  
 CAATGAAGGA AACTAAGAGA CAGTTACCAT CAACAGGTGA AACAGCTAAC  
 1060 1070 1080 1090 1100  
 CCATTCTTCA CAGCGGCAGC CCTTACTGTT ATGGCAACAG CTGGAGTAGC  
 1110 1120 1130  
 AGCAGTTGTA AAACGCAAAG AAGAAAACTA A

8. The DNA sequence according to Claim 6 which is the following sequence:

10 20 30 40 50  
 GAATTCGGT ACCATCAACA GGTGAAACAG CTAACCCATT CTTACACGCG  
 60 70 80 90 100  
 GCAGCCCTTA CTGTTATGGC AACAGCTGGA GTAGCAGCAG TTGTAACGCG  
 5  
 110 120 130 140 150  
 CAAAGAAGAA AACTAAGCTA TCACTTTGTA ATACTGAGTG AACATCAAGA  
 160 170 180 190 200  
 GAGAACCAGT CGGTTCTCTC TTTTATGTAT AGAAGAATGA GGTAAAGGAG  
 10  
 210 220 230 240 250  
 AGGTCACAAA CTAAACAAGT CTTAAAAAGC TGACCTTTAC TCCTTTTGAT  
 260 270 280 290 300  
 TAACTATATA TAATAAAAAA ATTAGGAAAA TAATAGCACT ATTAATTTTC  
 15  
 310 320 330 340 350  
 TTTTTAATA AAATCAAGGA GTAGATAATG ACTAGACAAC AAACCAAGAA  
 360 370 380 390 400  
 AAATTATTCA CTACGGAAAC TAAAAACCGG TACGGCTTCA GTAGCCGTTG  
 20  
 410 420 430 440 450  
 CTTTGACCGT TTTGGGCGCA GGTTTTGCAA ACCAAACAAC AGTTAAGGCG  
 460 470 480 490 500  
 GAAGGGGCTA AAATTGATTG GCAAGAAGAG TATAAAAAGT TAGACGAAGA  
 25  
 510 520 530 540 550  
 TAATGCTAAA CTTGTTGAGG TTGTTGAAAC CACAAGTTTG GAAAACGAAA  
 560 570 580 590 600  
 AACTCAAGAG TGAGAATGAG GAGAATAAGA AAAATTAGA CAACTTAGC  
 30  
 610 620 630 640 650  
 AAAGAAAATC AAGGAAAGCT CGAAAAATTG GAGCTTGAAT ATCTCAAAAA  
 660 670 680 690 700  
 ATTAGATCAC GAGCACAAAG AGCACCAAAA AGAACAACAA GAACAAGAAG  
 35  
 710 720 730 740 750  
 AGCGACAAAA AAATCAAGAA CAATTAGAAC GTAAATACCA ACGAGAAGTA  
 760 770 780 790 800  
 GAAAAACGTT ATCAAGAACA ACTCCAAAAA CAACAACAAT TAGAAACAGA  
 40  
 810 820 830 840 850  
 AAAGCAAATC TCAGAAGCTA GTCGTAAGAG CCTAAGCCGT GACCTTGAAG  
 860 870 880 890 900  
 CGTCTCGTGC AGCTAAAAAA GACCTTGAAG CTGAGCACCA AAAACTTGAA  
 45  
 50  
 55

910 920 930 940 950  
 GCTGAGCACC AAAAAGCTTAA AGAAGACAAA CAAATCTCAG ACGCAAGTCG  
 960 970 980 990 1000  
 TCAAGGCCTA AGCCGTGACC TTGAAGCGTC TCGTGCAGCT AAAAAAGAGC  
 1010 1020 1030 1040 1050  
 TTGAAGCAAA TCACCAAAAA CTTGAAGCTG AGCACCAAAA ACTTAAAGAA  
 1060 1070 1080 1090 1100  
 GACAAACAAA TCTCAGACGC AAGTCGTCAA GGCCTAAGCC GTGACCTTGA  
 1110 1120 1130 1140 1150  
 AGCGTCTCGT GCAGCTAAAA AAGAGCTTGA AGCAAATCAC CAAAAAGTTG  
 1160 1170 1180 1190 1200  
 AAGCAGAAGC AAAAGCACTC AAAGAACAAT TAGCGAAACA AGCTGAAGAA  
 1210 1220 1230 1240 1250  
 CTTGCAAAAC TAAGAGCTGG AAAAGCATCA GACTCACAAA CCCCTGATAC  
 1260 1270 1280 1290 1300  
 AAAACCAGGA AACAAAGCTG TTCCAGGTAA AGGTCAAGCA CCACAAGCAG  
 1310 1320 1330 1340 1350  
 GTACAAAACC TAACCAAAAC AAAGCACCAA TGAAGGAAAC TAAGAGACAG  
 1360 1370 1380 1390 1400  
 TTACCATCAA CAGGTGAAAC AGCTAACCCA TTCTTCACAG CGGCAGCCCT  
 1410 1420 1430 1440 1450  
 TACTGTTATG GCAACAGCTG GAGTAGCAGC AGTTGTAAAA CGCAAAGAAG  
 1460 1470 1480 1490 1500  
 AAAACTAAGC TATCACTTTG TAATACTGAG TGAACATCAA GAGAGAACCA  
 1510 1520 1530 1540 1550  
 GTCGGTTCTC TCTTTTATGT ATAGAAGAAT GAGGTTAAGG AGAGGTCACA  
 1560 1570 1580 1590 1600  
 AACTAAACAA CTCTTAAAAA GCTGACCCTT ACTAATAATC GTCTTTGTTT  
 1610 1620 1630 1640 1650  
 TATAATGAAA ACATTAACGA AATAATTAT TAAGGAGAGA ATACTAATGA  
 1660 1670 1680 1690 1700  
 ATATTAGAAA TAAGATTGAA AATAGTAAAA CACTACTATT TACATCCCTT  
 1710 1720 1730 1740 1750  
 GTAGCCGTGG CTCTACTAGG AGCTACACAA CCAGTTTCAG CCGAAACGTA  
 1760 1770 1780  
 TACATCACGC AATTTTGACT GGTCTGGGGA ATTG

9. A DNA sequence hybridizing to a DNA sequence of any one of Claims 6 to 8 under conventional conditions and encoding a protein displaying the same binding properties as the proteins of any one of Claims 1 to 5.

10. A recombinant plasmid containing a DNA sequence of any one of Claims 6 to 9.

11. A host cell transformed with the recombinant plasmid of Claim 10.

12. A host cell according to Claim 11 which belongs to the species *Escherichia coli*.

13. A process for producing Protein H comprising cultivating a host cell according to Claim 11 or 12 under suitable conditions, accumulating the protein H in the culture and recovering it therefrom.

14. A reagent kit for binding, separation and identification of human immunoglobulin G characterized in that it comprises a protein according to any one of Claims 1 to 5.

15. A pharmaceutical composition comprising a protein according to any one of Claims 1 to 5 and optionally a pharmaceutically acceptable additive or carrier.

16. A process for preparing a pharmaceutical composition, characterized in that a protein according to any one of Claims 1 to 5 is mixed with pharmaceutically acceptable additives.

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F i g . 1 ( A )

MET THR ARG GLN GLN THR LYS LYS ASN TYR SER LEU ARG LYS LEU 10  
 LYS THR GLY THR ALA SER VAL ALA VAL ALA LEU THR VAL LEU GLY 20 30  
 ALA GLY PHE ALA ASN GLN THR THR VAL LYS ALA GLU GLY ALA LYS 40  
 ILE ASP TRP GLN GLU GLU TYR LYS LYS LEU ASP GLU ASP ASN ALA 50 60  
 LYS LEU VAL GLU VAL VAL GLU THR THR SER LEU GLU ASN GLU LYS 70  
 LEU LYS SER GLU ASN GLU GLU ASN LYS LYS ASN LEU ASP LYS LEU 80 90  
 SER LYS GLU ASN GLN GLY LYS LEU GLU LYS LEU GLU LEU ASP TYR 100  
 LEU LYS LYS LEU ASP HIS GLU HIS LYS GLU HIS GLN LYS GLU GLN 110 120  
 GLN GLU GLN GLU GLU ARG GLN LYS ASN GLN GLU GLN LEU GLU ARG 130  
 LYS TYR GLN ARG GLU VAL GLU LYS ARG TYR GLN GLU GLN LEU GLN 140 150  
 LYS GLN GLN GLN LEU GLU THR GLU LYS GLN ILE SER GLU ALA SER 160  
 ARG LYS SER LEU SER ARG ASP LEU GLU ALA SER ARG ALA ALA LYS 170 180  
 LYS ASP LEU GLU ALA GLU HIS GLN LYS LEU GLU ALA GLU HIS GLN 190



## F i g . 1 ( B )

200 LYS LEU LYS GLU ASP LYS GLN ILE SER ASP ALA SER ARG GLN GLY 210  
 LEU SER ARG ASP LEU GLU ALA SER ARG ALA ALA LYS LYS GLU LEU 220  
 GLU ALA ASN HIS GLN LYS LEU GLU ALA GLU HIS GLN LYS LEU LYS 240  
 GLU ASP LYS GLN ILE SER ASP ALA SER ARG GLN GLY LEU SER ARG 250  
 ASP LEU GLU ALA SER ARG ALA LYS LYS GLU LEU GLU ALA ASN 270  
 HIS GLN LYS LEU GLU ALA GLU ALA LYS ALA LEU LYS GLU GLN LEU 280  
 ALA LYS GLN ALA GLU GLU LEU ALA LYS LEU ARG ALA GLY LYS ALA 300  
 SER ASP SER GLN THR PRO ASP THR LYS PRO GLY ASN LYS ALA VAL 310  
 PRO GLY LYS GLY GLN ALA PRO GLN ALA GLY THR LYS PRO ASN GLN 330  
 ASN LYS ALA PRO MET LYS GLU THR LYS ARG GLN LEU PRO SER THR 340  
 GLY GLU THR ALA ASN PRO PHE PHE THR ALA ALA ALA LEU THR VAL 360  
 MET ALA THR ALA GLY VAL ALA ALA VAL VAL LYS ARG LYS GLU GLU 370  
 ASN

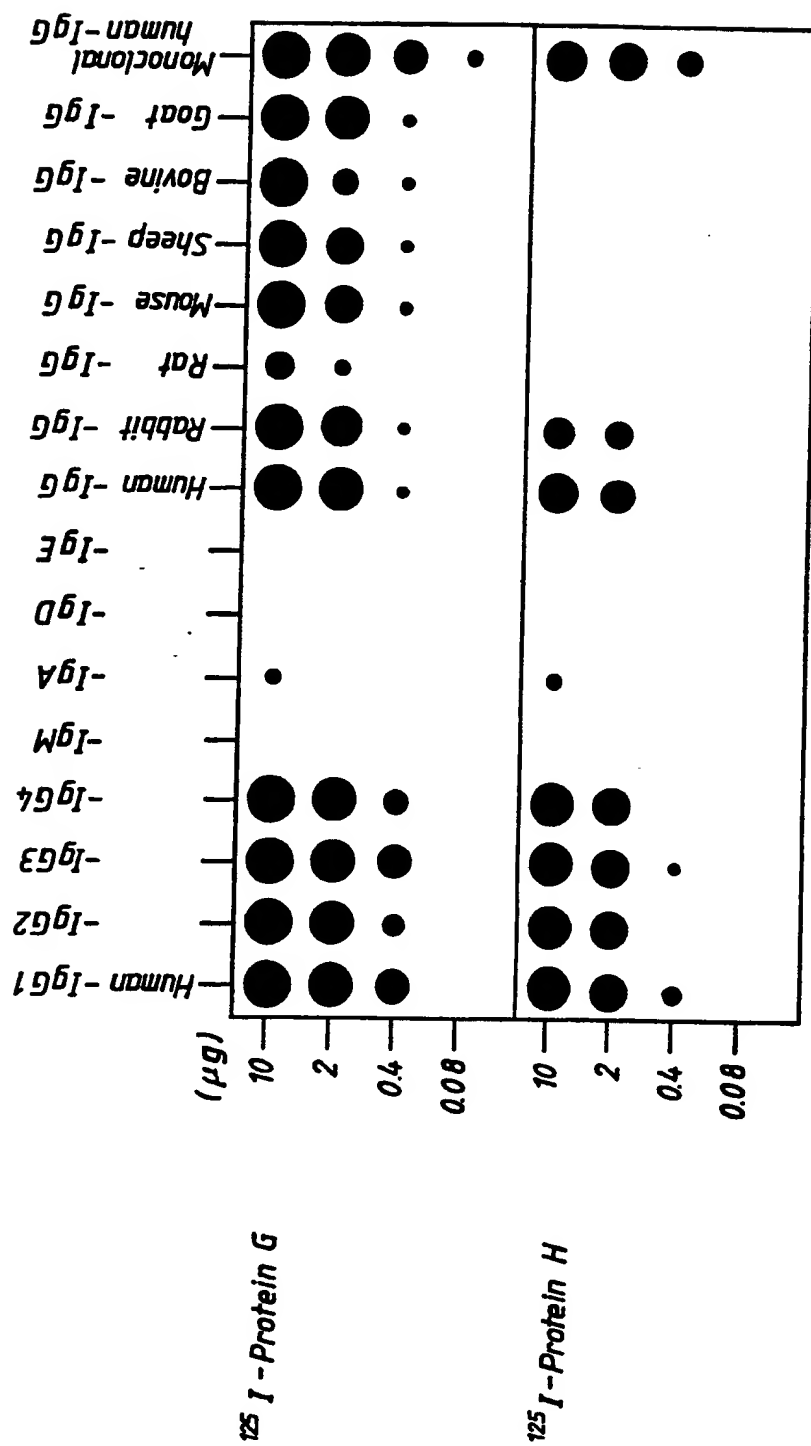
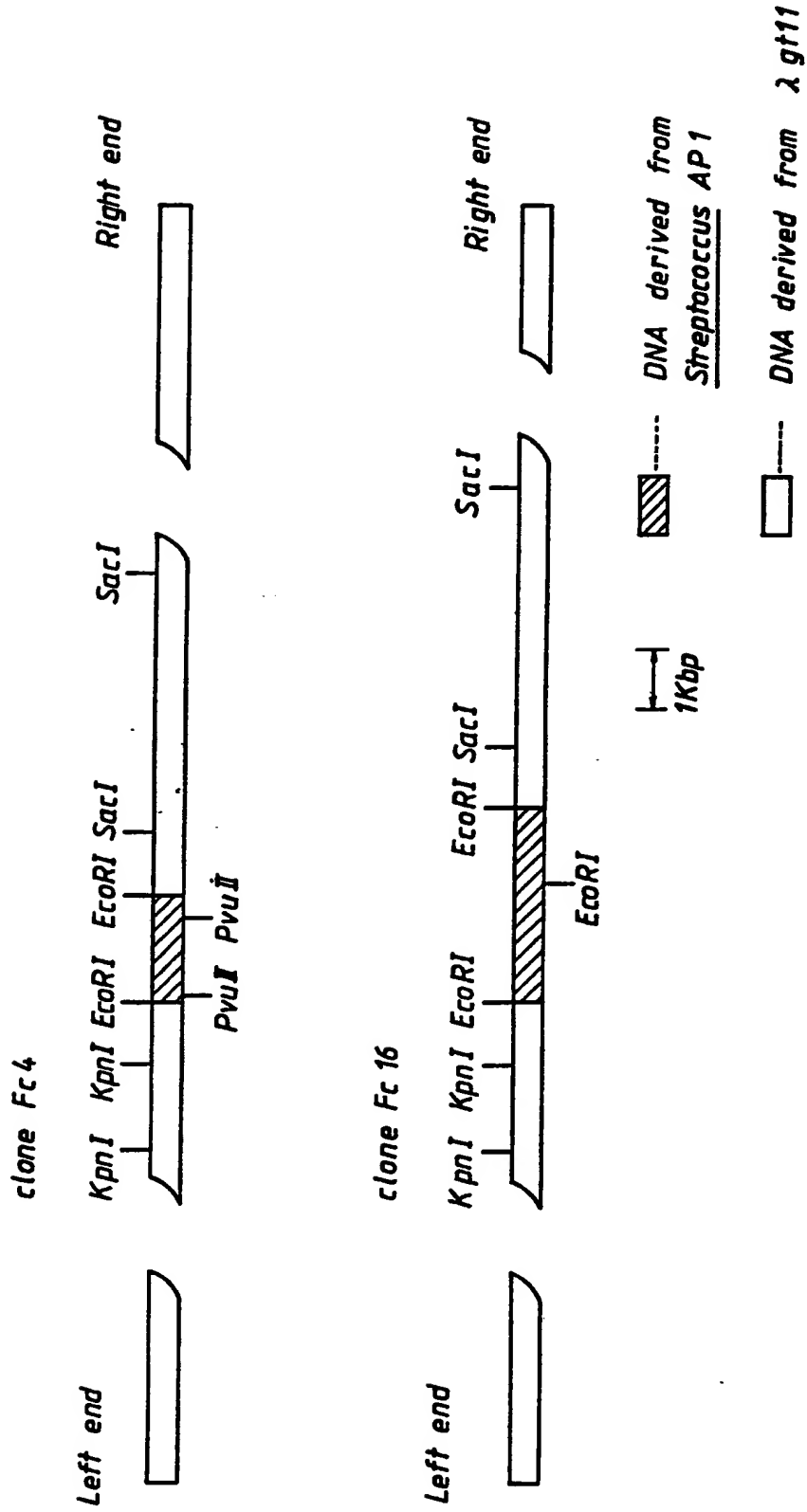


Fig. 2

Fig.3



## F i g . 4 ( A )

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10      20      30      40      50      60
GAATTCGTT ACCATCAACA GGTGAAACAG CTAACCCATT CTTACAGCG GCAGCCCTTA

70      80      90     100     110     120
CTGTTATGCG AACAGCTGGA GTAGCAGCAG TTGTAAACG CAAAGAAGAA AACTAAGCTA

130     140     150     160     170     180
TCACITTTGTA ATACTGAGTG AACATCAAGA GAGAACCAGT CGGTTCTCTC TTTTATGTAT

190     200     210     220     230     240
AGAAGAATGA GGTTAAGGAG AGGTCACAAA CTAACAACACT CTTAAAAAGC TGACCTTTAC

250     260     270     280     290     300
TCCITTTTGAT TAACTATATA TAATAAAAT ATTAGGAAA TAATAGCACT ATTAATTTTC

310     320     330     340     350     360
TTTTTTAATA AATCAAGGA GTAGATAATG ACTAGACAAC AAACCAAGAA AATTATTCA

370     380     390     400     410     420
CTACGGAAC TAAAAACCGG TACGGCTTCA GTAGCCGTTG CTTTGACCGT TTTGGCGCA

430     440     450     460     470     480
GGTTTTGCCA ACCAAACAAC AGTTAAGCGG GAAGGGGCTA AAATTGATTG GCAAGAAGAG

490     500     510     520     530     540
TATAAAAAGT TAGACGAAGA TAATGCTAAA CTTGTTGAGG TTGTTGAAAC CACAAGTTTG

550     560     570     580     590     600
GAAACGAAA AACTCAAGAG TGAGAAATGAG GAGAATAAGA AAAATTTAGA CAAACTTAGC

610     620     630     640     650     660
AAAGAAAATC AAGGAAAGCT CGAAAAATTG GAGCTTGACT ATCTCAAAA ATTAGATCAC

670     680     690     700     710     720
GAGCACAAG AGCACCAAAA AGAACACAA GAAACAAGAAG AGCGACAAA AAATCAAGAA

730     740     750     760     770     780
CAATTAGAAC GTAATACCA ACGAGAAGTA GAAAAACGTT ATCAAGAAC ACTCCAAAA

790     800     810     820     830     840
CAACAACAAT TAGAAACAGA AAAGCAAATC TCAGAAGCTA GTCGTAAGAG CCTAAGCCGT

850     860     870     880     890     900
GACCTTGAAG CGTCTCGTGC AGCTAAAAA GACCTTGAAG CTGAGCACCA AAAACTTGAA

```

## F i g . 4 ( B )

910	920	930	940	950	960
GCTGAGCACC	AAAACTTAA	AGAAGACAA	CAATCTCAG	ACGCAAGTCG	TCAAGGCCTA
970	980	990	1000	1010	1020
AGCCGTGACC	TTGAAGCGTC	TCGTGCAGCT	AAAAAAGAGC	TTGAAGCAAA	TCACCAAAAA
1030	1040	1050	1060	1070	1080
CTTGAAGCTG	AGCACCAGAA	ACTTAAAGAA	GACAAACAAA	TCTCAGACGC	AAGTCGTCAA
1090	1100	1110	1120	1130	1140
GGCCTAAGCC	GTGACCTTGA	AGCGTCTCGT	GCAGCTAAAA	AAGAGCTTGA	AGCAAAATCAC
1150	1160	1170	1180	1190	1200
CAAAAACCTG	AAGCAGAAGC	AAAGCACTC	AAAGAACAAT	TAGCGAAACA	AGCTGAAGAA
1210	1220	1230	1240	1250	1260
CTTGCAAAAC	TAAGAGCTGG	AAAAGCATCA	GACTCACAAA	CCCCTGATAC	AAAACCACGA
1270	1280	1290	1300	1310	1320
AACAAAGCTG	TTCCAGGTAA	AGGTCAAGCA	CCACAAGCAG	GTACAAAACC	TAACCACAAAC
1330	1340	1350	1360	1370	1380
AAAGCACCAA	TGAAGGAAC	TAAGAGACAG	TTACCATCAA	CAGGTGAAC	AGCTAACCCA
1390	1400	1410	1420	1430	1440
TTCTTCACAG	CGGCAGCCCT	TACTGTTATG	GCAACAGCTG	GAGTAGCAGC	AGTTGTAAAA
1450	1460	1470	1480	1490	1500
CGCAAAGAAG	AAACTAAGC	TATCAGTTTG	TAATACTGAG	TGAACATCAA	GAGAGAACCA
1510	1520	1530	1540	1550	1560
GTCGGTTCTC	TCTTTTATGT	ATAGAAGAAT	GAGGTTAAGG	AGAGGTCACA	AACTAAACAA
1570	1580	1590	1600	1610	1620
CTCTTAAAAA	GCTGACCTTT	ACTAATAATC	GTCTTTGTTT	TATAATGAAA	ACATTAACGA
1630	1640	1650	1660	1670	1680
AATAATTAT	TAAGGAGAGA	ATACTAATGA	ATATTAGAAA	TAAAGATTGAA	AATAGTAAAA
1690	1700	1710	1720	1730	1740
CACTACTATT	TACATCCCTT	GTAGCCGTGG	CTCTACTAGG	AGCTACACAA	CCAGTTTCAG
1750	1760	1770	1780		
CCGAAACGTA	TACATCACGC	AATTTTGACT	GGTCTGGGGA	ATTC	

## F i g . 5 ( A )

10 20 30 40 50 60  
 ATGACTAGACAAACCAAGAAATATTATTCACTACGGAACCTAAAAACCGGTACGGCT  
 MetThrArgGlnGlnThrLysLysAsnTyrSerLeuArgLysLeuLysThrGlyThrAla

70 80 90 100 110 120  
 TCAGTAGCCGTTGCTTTGACCGTTTTGGCGCAGGTTTTTGCAAAACCAACACAGTTAAG  
 SerValAlaValAlaLeuThrValLeuGlyAlaGlyPheAlaAsnGlnThrThrValLys

130 140 150 160 170 180  
 QCGGAAGGGGCTAAATTTGATTGGCAAGAAGAGTATAAAAAGTTAGACGAAGATAATGCT  
 AlaGluGlyAlaLysIleAspTrpGlnGluGluTyrLysLysLeuAspGluAspAsnAla

190 200 210 220 230 240  
 AAACCTTGTTGAGGTTGTTGAAACCACAAGTTTGGAAAACGAAAACTCAAGAGTGAAGAT  
 LysLeuValGluValValGluThrThrSerLeuGluAsnGluLysLeuLysSerGluAsn

250 260 270 280 290 300  
 GAGGAGAATAAGAAAAATTTAGACAAACTTAGCAAAGAAAATCAAGGAAAGCTCGAAAAA  
 GluGluAsnLysLysAsnLeuAspLysLeuSerLysGluAsnGlnGlyLysLeuGluLys

310 320 330 340 350 360  
 TTGGAGCTTGACTATCTCAAAAATTTAGATCAGGAGCACAAGAGCACCACCAAAAGAACAA  
 LeuGluLeuAspTyrLeuLysLysLeuAspHisGluHisLysGluHisGlnLysGluGln

370 380 390 400 410 420  
 CAAGAACAAGAGAGCGACAAAAAATCAAGAACAATTAGAACGTAAATACCAACGAGAA  
 GlnGluGlnGluGluArgGlnLysAsnGlnGluGlnLeuGluArgLysTyrGlnArgGlu

430 440 450 460 470 480  
 GTAGAAAAACGTTATCAAGAACAACCTCCAAAAACCAACAATTAGAAAAACAGAAAGCAA  
 ValGluLysArgTyrGlnGlnGlnLeuGlnLysGlnGlnGlnLeuGluThrGluLysGln

490 500 510 520 530 540  
 ATCTCAGAAGCTAGTCGTAAGAGCCTAAGCCGTGACCTTGAAGCGTCTCGTGCAGCTAAA  
 IleSerGluAlaSerArgLysSerLeuSerArgAspLeuGluAlaSerArgAlaAlaLys

550 560 570 580 590 600  
 AAAGACCTTGAAGCTGAGCACCACCAAACTTGAAGCTGAGCACCACCAAACTTAAAGAAGAC  
 LysAspLeuGluAlaGlnHisGlnLysLeuGluAlaGluHisGlnLysLeuLysGluAsp

## F i g . 5 ( B )

610 620 630 640 650 660  
 AAACAAATCTCAGACGCAAGTCGTCAAGGCCTAAGCCGTGACCTTGAAGCGTCTCGTGCA  
 LysGlnIleSerAspAlaSerArgGlnGlyLeuSerArgAspLeuGluAlaSerArgAla

670 680 690 700 710 720  
 GCTAAAAAGAGCTTGAAGCAAAATCACCAAAAACCTTGAAGCTGAGCACCAAAAACCTTAAA  
 AlaLysLysGluLeuGluAlaAsnHisGlnLysLeuGluAlaGluHisGlnLysLeuLys

730 740 750 760 770 780  
 GAAGACAAACAAATCTCAGACGCAAGTCGTCAAGGCCTAAGCCGTGACCTTGAAGCGTCT  
 GluAspLysGlnIleSerAspAlaSerArgGlnGlyLeuSerArgAspLeuGluAlaSer

790 800 810 820 830 840  
 CGTGCAGCTAAAAAGAGCTTGAAGCAAAATCACCAAAAACCTTGAAGCAGCAAGCAAAAGCA  
 ArgAlaAlaLysLysGluLeuGluAlaAsnHisGlnLysLeuGluAlaGluAlaLysAla

850 860 870 880 890 900  
 CTCAAAGAACAAATTAGCGAAACAAGCTGAAGAACTTGCAAAAACCTAAGAGCTGGAAAAGCA  
 LeuLysGluGlnLeuAlaLysGlnAlaGluGluLeuAlaLysLeuArgAlaGlyLysAla

910 920 930 940 950 960  
 TCAGACTCACAAACCCCTGATACAAACCCAGGAACAAGCTGTTCCAGGTAAGGTCAA  
 SerAspSerGlnThrProAspThrLysProGlyAsnLysAlaValProGlyLysGlyGln

970 980 990 1000 1010 1020  
 GCACCACAAAGCAGGTACAAAACCTAACCAAAACAAGCACCAATGAAGGAACTAAGAGA  
 AlaProGlnAlaGlyThrLysProAsnGlnAsnLysAlaProMetLysGluThrLysArg

1030 1040 1050 1060 1070 1080  
 CAGTTACCATCAACAGGTGAACACAGCTAACCCATTCTTCACAGCGCAGCCCTTACTGTT  
 GlnLeuProSerThrGlyGluThrAlaAsnProPheThrAlaAlaAlaLeuThrVal

1090 1100 1110 1120 1130  
 ATGGCAACAGCTGGAGTAGCAGCAGTTGTAAAACGCAAAAGAAAGAACTAA  
 MetAlaThrAlaGlyValAlaAlaValValLysArgLysGluGluAsn\*\*\*

Fig. 6

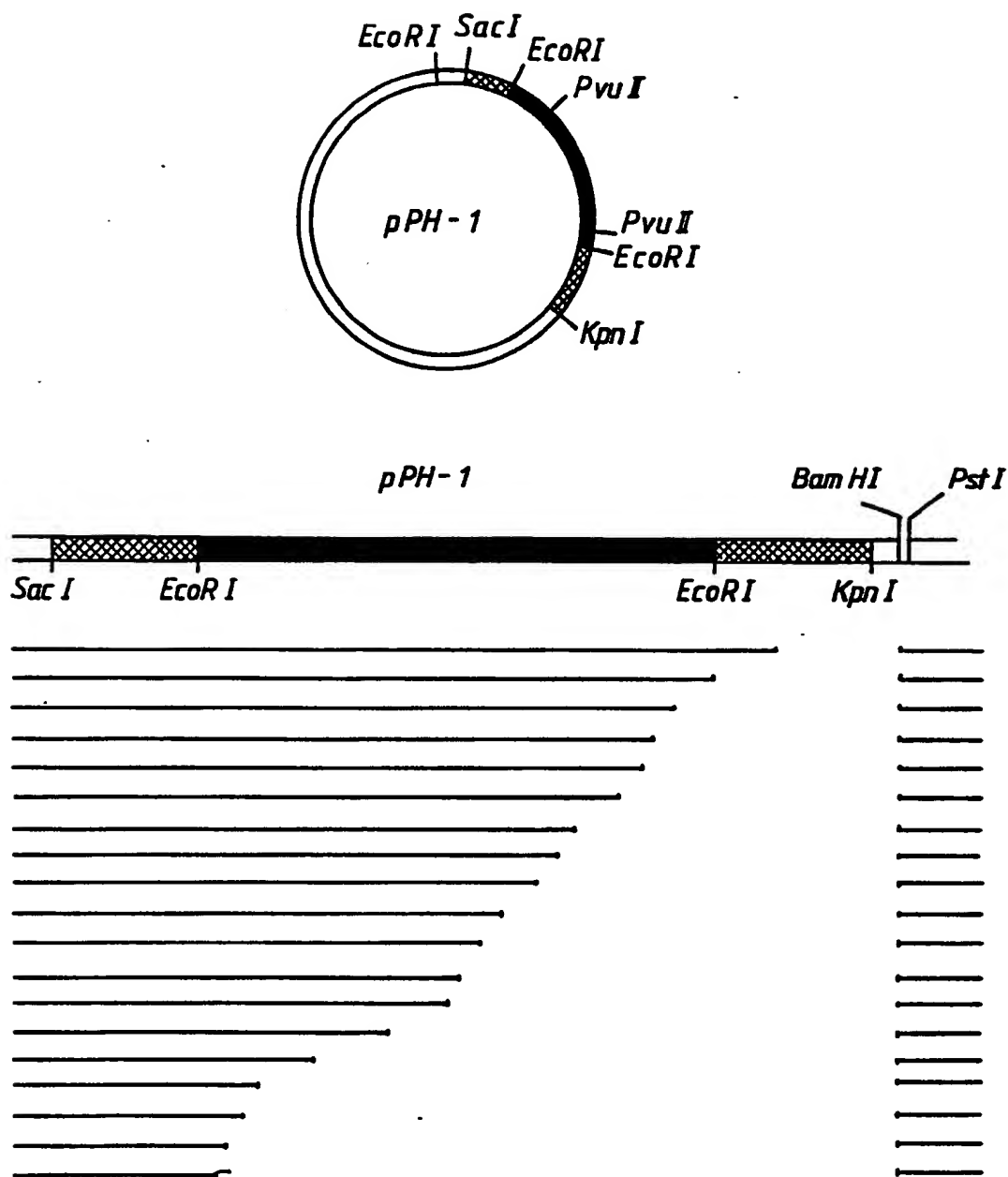
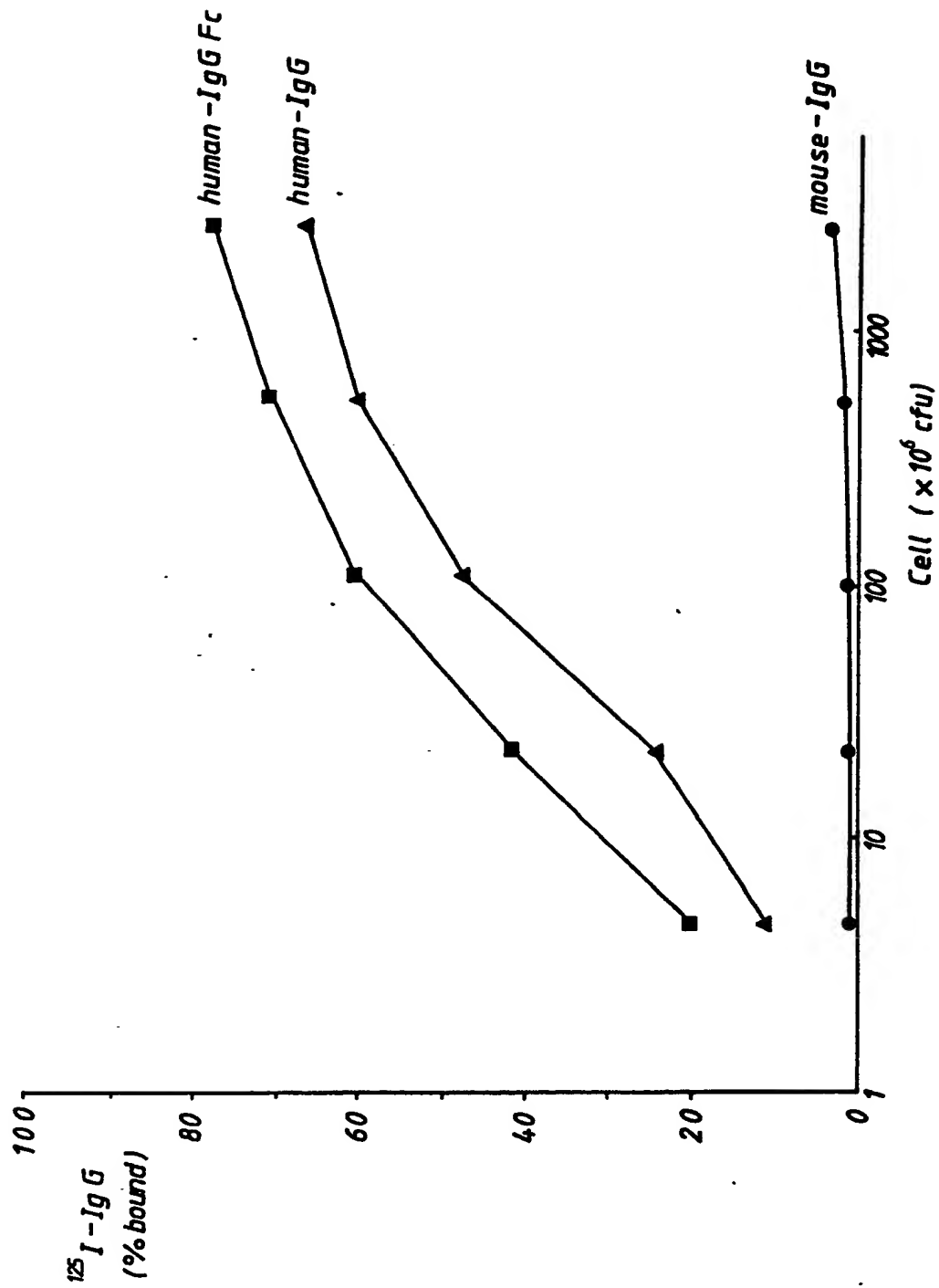
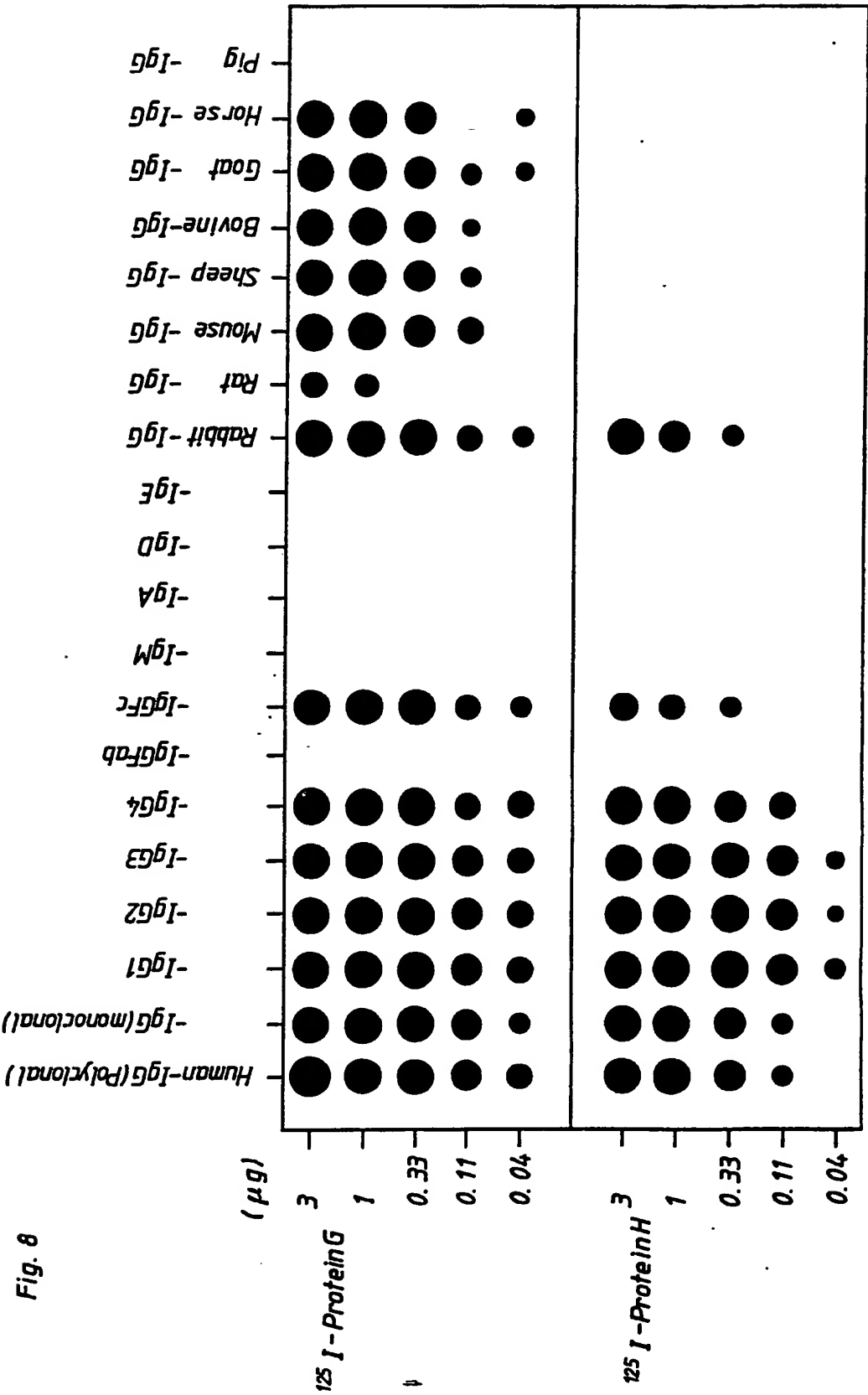




Fig. 7







European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number

EP 89 11 3430

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	CHEMICAL ABSTRACTS, vol. 96, 10th May 1982, page 580, abstract 160649d, Columbus, Ohio, US; A. GRUBB et al.: "Isolation and some properties of an IgG Fc-binding protein from group A streptococci type 15", & INT. ARCH. ALLERGY APPL. IMMUNOL. 1982, 67(4), 369-76 * Abstract * ---	1,3,14-16	C 07 K 13/00 C 12 N 15/31 G 01 N 33/566 A 61 K 37/02
X	THE JOURNAL OF IMMUNOLOGY, vol. 138, no. 3, 1st February 1987, pages 922-926, The American Association of Immunologists; F.A. NARDELLA et al.: "T15 group a streptococcal Fc receptor binds to the same location on IgG as staphylococcal protein A and IgG rheumatoid factors" * Page 923, column 1, lines 10-16 * ---	1,3,14-16	
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P,X O	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE USA, vol. 86, June 1989, pages 4741-4745; D.G. HEATH et al.: "Fc-receptor and M-protein genes of group A streptococci are products of gene duplication" * Whole document especially page 4741, column 1: "Footnotes" * --- -/-	1,3,6,9-13	
The present search report has been drawn up for all claims			
Place of search <b>THE HAGUE</b>		Date of completion of the search <b>26-02-1990</b>	Examiner <b>SKELLY J.M.</b>
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons</p> <p>&amp; : member of the same patent family, corresponding document</p>			

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
A	BIOTECHNOLOGY, vol. 5, July 1987, pages 697-703; M.D.P. BOYLE et al.: "Bacterial Fc receptors" * Pages 699-700 * -----		
			TECHNICAL FIELDS SEARCHED (Int. Cl. 5)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 26-02-1990	Examiner SKELLY J.M.
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document			